

- receptive fields with microstimulation of frontal cortex. *Neuron* 50, 791–798.
7. Moore, T., and Fallah, M. (2004). Microstimulation of the frontal eye field and its effects on covert spatial attention. *J. Neurophysiol.* 91, 152–162.
 8. Moore, T., and Armstrong, K.M. (2003). Selective gating of visual signals by microstimulation of frontal cortex. *Nature* 421, 370–373.
 9. Ruff, C.C., Blankenburg, F., Bjoertomt, O., Bestmann, S., Freeman, E., Haynes, J., Rees, G., Josephs, O., Deichmann, R., and Driver, J. (2006). Frontal influences of human retinotopic visual cortex revealed by concurrent TMS-fMRI and psychophysics. *Curr. Biol.* 16, 1479–1488.
 10. Schall, J.D., and Thompson, K.G. (1999). Neural selection and control of visually guided eye movements. *Annu. Rev. Neurosci.* 22, 241–259.
 11. Bruce, C.J. (1990). Integration of sensory and motor signals for saccadic eye movements in the primate frontal eye fields. In *Signals and Senses, Local and Global Order in Perceptual Maps*, G.M. Edelman, W.E. Gall, and W.M. Cowan, eds. (New York: Wiley), pp. 261–314.
 12. Thompson, K.G., Biscoe, K.L., and Sato, T.R. (2005). Neuronal basis of covert spatial attention in the frontal eye field. *J. Neurosci.* 25, 9479–9487.
 13. Moore, T., Armstrong, K.M., and Fallah, M. (2003). Visuomotor origins of covert spatial attention. *Neuron* 40, 671–683.
 14. Barone, P., Batardiere, A., Knoblauch, K., and Kennedy, H. (2000). Laminar distribution of neurons in extrastriate areas projecting to visual areas V1 and V4 correlates with the hierarchical rank and indicates the operation of a distance rule. *J. Neurosci.* 20, 3263–3281.
 15. Schall, J.D., Morel, A., King, D.J., and Bullier, J. (1995). Topography of visual cortex connections with frontal eye field in macaque: convergence and segregation of processing streams. *J. Neurosci.* 15, 4464–4487.
 16. Winkowski, D.E., and Knudsen, E.I. (2006). Top-down gain control of the auditory space map by gaze control circuitry in the barn owl. *Nature* 439, 336–339.

MPI for Biological Cybernetics,
Spemannstrasse 38, 72076 Tuebingen,
Germany.
E-mail: Nikos.Logothetis@tuebingen.mpg.de

DOI: 10.1016/j.cub.2006.07.009

Engineered Crops: Transgenes Go Wild

Genetically modified *Agrostis stolonifera* has escaped from cultivation. For the first time, a herbicide-resistant perennial weed has established itself in wild populations.

Eric J. Baack

A decade after their commercial introduction, transgenic crops are widely planted in the United States. Thus far, the feared ecological consequences have not materialized. Cotton engineered with the Cry1A toxin from the bacterium *Bacillus thuringiensis* still kills pink bollworms [1]. Weedy relatives of crops have not become significantly more difficult to manage as a result of transgene escape [2]. This may change. For the first time, transgenic juvenile plants of a perennial weed have been found in the wild [3].

In 2003, transgenic creeping bentgrass, *Agrostis stolonifera* L., was propagated in central Oregon, USA. Designed to help golf course managers to keep putting greens free of weeds, RoundUp Ready® creeping bentgrass (Scotts Company, Gervais, Oregon, USA and Monsanto Company, St. Louis, Missouri, USA) was designed to allow the use of the popular herbicide glyphosphate while keeping the turf intact. The seed production took place on 162 hectares within a 4453

hectare control district where only transgenic bentgrass could be grown, and all other bentgrass had to be removed during propagation and in the following years. Harvested seeds were transported in sealed containers, and combines were fumigated prior to leaving the control area [4]. Despite these measures, the wind-pollinated flowers made escape of the transgene through pollen likely.

In the years following propagation, Reichman *et al.* [3] surveyed potential bentgrass habitat on public lands up to 4.8 kilometers downwind of the control district and sampled 20,400 juvenile *Agrostis* plants. They first used an immunological test to screen 40–50 individuals at a time for the presence of the 5-enol-pyruvylshikimate-3-phosphate (EPSPS) protein from the *Agrobacterium* strain CP4, which confers glyphosphate resistance in the engineered variety. When a bulk sample tested positive, individual plants were then verified as transgenic by polymerase chain reaction (PCR) amplification of the CP4 EPSPS coding region.

Reichman *et al.* [3] found transgenic plants up to 3.8 kilometers from the experimental zone, not only among existing naturalized and native populations of creeping bentgrass, but also in isolation. Analysis of two molecular markers, ITS and *matK*, from the transgenic plants verified that they were *Agrostis stolonifera* and not hybrids between *A. stolonifera* and other *Agrostis* species. The molecular markers could distinguish hybrids, but were unable to differentiate between cultivated and naturalized populations of *A. stolonifera*. Transgenic plants found near naturalized *A. stolonifera* were presumed to be the result of pollen dispersal. Herbicide-resistant transgenic plants found up to 1 kilometer from the nearest experimental field — and near *A. gigantea* — were likely due to seed dispersal. Finding escaped transgenic plants was not a surprise. An earlier study set the stage by documenting the spread of pollen up to 21 kilometers from the site planted in transgenic bentgrass [5]. The spread of transgenes into natural areas has occurred.

Transgenes were found in just nine of the 20,400 plants sampled in the study (0.04%). This should not be taken as an absolute number of escaped plants: Reichman *et al.* [3] surveyed only the publicly owned portion of the suitable habitat. Ninety percent of the potential habitat occurs on private land.

The spread by seed documented in this study is an important reminder of the difficulty of containing crop alleles. Seeds lost during harvest have allowed genetically engineered corn and canola to appear in agricultural fields one or more years following the planting of transgenic crops [2,6], and canola seeds lost by trucks often germinate along roadsides. In this study, creeping bentgrass seeds dispersed by wind: even great care in seed handling will not prevent the spread of seeds from some genetically modified crops. The engineering of transgenes into organelles has been proposed to reduce the risk of transgene escape [7]. Organelles typically have maternal inheritance and so are not transmitted by pollen. This technology should greatly reduce the spread of transgenes, but some escape via seeds will remain likely, particularly in crops with very small wind-dispersed seeds.

What effect, if any, will these escaped transgenes have in the wild environment? The concern is that transgenes escaping from crops could create new problem weeds: plants resistant to insects due to *B. thuringiensis* transgenes might be able to out-compete other plants, while herbicide resistance might hinder their control. This danger is not unique to transgenes: gene flow from other crops, including sorghum and canola, has led to the development of more problematic weeds [8], and traits such as herbicide resistance have been bred into crops using traditional techniques. The particular risks from *Agrostis stolonifera* appear limited: it is listed as a weed in some states [9], but it is not likely to pose a problem to farmers beyond those producing grass seed. Although *A. stolonifera* has invaded wetlands and grasslands in many states, its effects on ecosystems are considered limited [10].

While herbicide-resistant creeping bentgrass may be benign in the wild, it will provide essential information on the fate of transgenes and other crop alleles

in wild environments. Because transgenes are introduced from distant organisms — from a bacterium in this case — they can be readily tracked in the environment. Non-transgenic crop alleles also escape into wild populations, but because of limited knowledge of the molecular basis of most crop traits and the frequency of these alleles in wild populations, tracing non-transgenic crop alleles and their effects poses a more difficult challenge. It may also be easier to understand the phenotypic and ecological effects of transgenes compared to other crop alleles. Genetic engineers insert alleles that minimize pleiotropic effects, while traditional plant breeders have fewer choices. Thus, transgenes may provide insights into the movement and effects of crop alleles in wild populations that could not be obtained previously.

The perennial habit of creeping bentgrass raises the possibility that transgene movement might persist for many years in wild populations, even if the transgene poses a significant cost to the plant in the absence of glyphosphate use. Herbicide resistance often carries a cost, but not always [11]. The transgene should increase in frequency if bentgrass is treated with glyphosphate. Glyphosphate is one of the few herbicides licensed for use along waterways, where creeping bentgrass habitat occurs in central Oregon.

Reichman *et al.* [3] have initiated an important line of research, for very little is known about the effects of potentially beneficial crop alleles in wild populations. Previous work on sunflowers suggests that some crop alleles could have significant effects on plant fitness. Hybrids between the transgenic sunflowers producing *B. thuringiensis* toxins and wild plants produced more seeds, potentially leading to greater problems for farmers in areas where weedy sunflowers regularly invade fields [12]. However, key questions about the long term effects of

transgenes in wild populations were never answered; with the withdrawal of *B. thuringiensis* sunflowers from field trials, all research ended. The escaped transgenic bentgrass will offer important clues as to what might happen as more engineered crops encounter their wild relatives.

References

1. Tabashnik, B.E., Dennehy, T.J., and Carriere, Y. (2005). Delayed resistance to transgenic cotton in pink bollworm. *Proc. Natl. Acad. Sci. USA* 102, 15389–15393.
2. Legere, A. (2005). Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L.) as a case study. *Pest. Manag. Sci.* 61, 292–300.
3. Reichman, J.R., Watrud, L.S., Lee, E.H., Burdick, C.A., Bollman, M.A., Storm, M.J., King, G.A., and Mallory-Smith, C. (2006). Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in non-agronomic habitats. *Mol. Ecol.*, in press.
4. Department of Agriculture (2002). Oregon Administrative Rule 603-052-1240.
5. Watrud, L.S., Lee, E.H., Fairbrother, A., Burdick, C., Reichmann, J.R., Bollman, M., Storm, M., King, G., and Van de Water, P. (2004). Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proc. Natl. Acad. Sci. USA* 101, 14533–14538.
6. USDA APHIS (2002). USDA investigates biotech company for possible permit violations. <http://www.aphis.usda.gov/lpa/news/2002/11/prodigene.html>.
7. Daniell, H., Khan, M.S., and Allison, L. (2002). Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology. *Trends Plant Sci.* 7, 84–91.
8. Ellstrand, N.C., Prentice, H.C., and Hancock, J.F. (1999). Gene flow and introgression from domesticated plants into their wild relatives. *Annu. Rev. Ecol. Syst.* 30, 539–563.
9. USDA, NRCS (2006). The PLANTS Database (<http://plants.usda.gov>, 30 May 2006). National Plant Data Center, Baton Rouge, LA, USA.
10. California Invasive Plant Council (2006). California Invasive Plant Inventory, California Invasive Plant Council, Berkeley, CA, USA.
11. Snow, A.A., Andersen, B., and Jorgenson, R.B. (1999). Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. *Mol. Ecol.* 8, 605–615.
12. Snow, A.A., Pilson, D., Rieseberg, L.H., Pleskac, N., Reagon, M.R., Wolf, D.E., and Selbo, S.M. (2003). A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecol. Appl.* 13, 279–286.

Department of Biology, Indiana University, 1001 E 3rd St, Bloomington, Indiana 47405, USA.
E-mail: ebaack@indiana.edu